IN THE SPECIFICATION

Responding to requests of Examiner Nashed

Please substitute the paragraph, which begins on page 2, line 23, of the specification, following the heading "SUMMARY OF THE INVENTION" with the following paragraph:

In accordance with the present invention, there is provided a method for producing a semi-synthetic fusion protein *in vitro,* comprising the steps of producing a target protein fused to a protein splicing element (an intein) and selectively cleaving the fusion and ligating a synthetic protein or peptide at the C-terminal thioester of the target protein, which overcome many of the disadvantages and problems noted above. The term "protein splicing element" according to U.S. Patent 5,834,247 ('247) is intended to include native and modified protein splicing elements, where modification of the protein splicing element may include: a mutation of one or more amino acid residues at the splice junction; or derivatives that are exemplified by the introduction of a protein phosphorylation, glycosylation or photolysis activation site at the sequence surrounding the mutation, or chemical modification of splice junction residues. Specifically, the present invention has higher yields due to better thiol-induced cleavage with thiol reagents which have been optimized for the ligation reaction. Off-column ligation allows for sample concentration as well as the use of less peptide. In a particularly preferred embodiment, thiol reagents such as 2-mercaptoethanesulfonic acid (MESNA), which is an odorless thiol-reagent, is used for cleavage and ligation along with the Mxe Gyr A intein, which is from

a bacterial source and often expresses better in bacterial cells. Furthermore, the present invention allows peptides to be directly ligated to the thioester bond formed between an intein and the target protein. The present invention also provides a method for producing a cytotoxic protein, comprising the steps of producing a truncated, inactive form of the protein *in vivo* which is fused to a protein splicing element, and selectively cleaving the fusion and ligating a synthetic protein or peptide at a C-terminal thioester of the target protein to restore the activity of the native cytotoxic protein. Recombinant vectors for producing such cleavable fusion proteins are also provided.

Please substitute two successive paragraphs, the first of which begins on page 7, line 5, of the specification, with the following two paragraphs:

The ligation procedure disclosed herein utilizes a protein splicing element, an intein (Perler, et al., (1994) *Nucleic Acids Res.* 22:1125-1127) to precisely create a thioester at the ~~C-terminal α-carbon~~ C-terminus of an expressed protein. This reactive thioester could be present between the target protein and intein or generated by the addition of a thiol reagent. Previously the generation of such a thioester was described using an intein (ClVPS) that was modified to undergo thiol inducible cleavage at its N-terminal junction in the presence of the thiol reagent dithiothreitol (DTT) (Chong, et al. (1997) *supra*; Comb, et.al. U.S. Patent No. 5,496,714). This C-terminal thioester was previously used in a 'native chemical ligation' type reaction (Dawson, et al., (1994) *Science* 266:776-779) to fuse ^{35}S-cysteine or a peptide fragment

containing an N-terminal cysteine to a bacterially expressed protein (Example 19, Comb, et.al. U.S. Patent No. 5,834,247, Chong (1996) *supra* and Chong (1997) *supra.*

The ligation method of the instant invention begins with the purification of the thioester-tagged target protein using an intein as described (Chong, et.al. (1997) *supra*). The direct ligation method of the instant invention begins with the isolation of a precursor composed of the target protein-intein-CBD. In one preferred embodiment, the host cell is bacterial. In other embodiments the host cell may be yeast, insect, or mammalian. A cysteine thiol at the N-terminus of a synthetic peptide nucleophilicly attacks a C-terminal thioester present on the freshly isolated ~~C-terminal α-carbon of the~~ target protein or directly attacks the thioester present between the target protein and intein. This initially generates a thioester between the two reactants which spontaneously rearranges into a native peptide bond (Figure 1).

Interview Summary

Applicants wish to express their appreciation for the opportunity of interviews in person and by telephone to discuss this application.

The present response and comments are in response to an office action prepared 4 months after an interview in person at the USPTO between applicants and Supervisory Examiner Achutermurthy and Examiner Moore on August 15, 2003. Examiner Nashed was unable to attend because of a personal emergency. At that interview, the present application 09/786,009 and a related application 09/249,543 were discussed together. An interview summary was provided in which the Examiner recognized that a proposed element (b) in independent claims 12, 22 ,25 and 28, specifying MESNA would distinguish the claimed invention from the cited prior art and that a provisional obviousness double patenting rejection would be reconsidered. As a result of the interview, applicants submitted a response on August 20, 2003, which addressed the issues raised at the interview.

On December 4, 2003, instead of a Notice of Allowability for complying with all outstanding issues raised by the Examiner, a 23-page office action was mailed to Applicants in which two new prior art references were introduced and a large number of new objections were raised under 35 U.S.C. §112 and 35 U.S.C. §103 in addition to new double patenting rejections.

A draft response was prepared and faxed to Examiner Moore prior to a telephone interview conducted with Examiner Moore and Examiner Nashid Nashed on February 24, 2004. Supervisory Examiner Achutermurthy was in

India for an extended period at the time of the telephone interview. The draft now serves as the basis for a formal response with some additional comments. Applicants have delayed in formalizing the response after the telephone interview on February 24, 2004 as it was deemed necessary to review the Examiner's pending office action (mailed in April 2004) on the related case (09/249,543) for which a response to the previous office action had also been mailed on August 20, 2003.

The following comments summarize the telephone interview and respond to the issues raised by Examiner Nashed in the presence of Examiner Moore.

During the interview, Examiner Nashed asserted that the use of MESNA was obvious in intein cleavage for the following reasons:

(a) Examiner Nashed asserted that he was an expert in thiol chemistry having conducted research in chemical cleavage of proteases. However, the Examiner acknowledged he had never experimented with intein mediated peptide ligation.

(b) In the opinion of Examiner Nashed, applicants use of MESNA in intein mediated peptide cleavage and ligation was obvious because he asserted that in his experience, all thiol reagents worked equally well in thiol reactions. Hence the Examiner asserted that the Burton reference rendered the present claimed invention obvious because Burton did not discriminate between thiol reagents and because Burton included MESNA in the list of 15 reagents described. In particular, the Examiner asserted that the fact that MESNA did not smell was a sufficient criterium for a skilled artisan to select

this thiol reagent over others merely by using a Dictionary of Organic Compounds.

Moreover, the Examiner did not discriminate between (a) free radical chemistry used in the reference and nucleophilic substitutions upon which the claimed invention relies nor (b) covalent attachment of a thioester to an ethylene to form a sorption product in the reference from the claimed invention which requires thiol induced cleavage of intein fusion products and protein ligation.

(c) Examiner Nashed rejected secondary considerations of (i) unexpected results and (ii) commercial success to support non-obviousness although Examiner Moore noted the rapid switch to MESNA by others in the field of intein mediated cleavage and ligation after the publication of the above claimed invention by the applicants (equivalent to commercial success).

Applicants assert that the Examiner Nashed's rejection of the claims on the basis of obviousness as stated in the telephone interview and repeated above are improper for reasons that include the following:

(a) Examiner Nashed has used a subjective standard of obviousness by using his own research experience as a guide to a determination of obviousness and has not applied the standard of objectivity required by MPEP 2141.03 which teaches that "the importance of resolving the level of ordinary skill in the art lies in the necessity of maintaining objectivity in the obviousness inquiry".

(b) The Examiner's assertion based on his own experience that all thiol reagents work equally well in all reactions teaches away from the claimed invention. The Examiner's assertion that the lack of smell was a sufficient and obvious criterium to select MESNA is not supported by the facts and contradicts applicants' findings concerning which thiol reagents were effective in nucleophilic substitution reactions for intein mediated cleavage suitable for ligation of a protein. In fact, DTT is odorless. Nonetheless, applicants searched and found thiol reagents that gave improved results over DTT. The assertion by the Examiner, that applicants merely open the Dictionary of Organic Compounds to select any thiol reagent that lacks a smell is an exercise in hindsight and is also not supported by the evidence in Figure 2 or throughout the above application. (see page 22 of this Response for a further discussion of the lack of bases for this rejection.)

Applicants provide evidence in Figure 2 of the above application that different thiol reagents work significantly differently in intein mediated cleavage and protein ligation reactions. For example, Figure 2 of the present application shows that the thiol reagent, 3-mercaptopropionic acid, was ineffective in intein mediated cleavage and ligation, while odorless DTT was partially effective but significantly less effective than thiophenol and MESNA. The use of the Burton reference by the Examiner to support the contention that all thiol reagents act similarly reinforces applicants assertion that Burton et al. teaches away from the present claimed invention. Moreover, Burton et al. has been improperly applied to the present claimed invention (see MPEP 1504) as the reference differs in scope and content from the claimed invention (see also page 20 of the present response).

(c) The Examiner's rejection of secondary considerations as evidence of non-obviousness is improper. MPEP 1503 states that "[One of][t]he basic factual enquiries guiding the evaluation of obviousness as outlined in the Supreme Court in Graham v. John Deere Co., 383 U.S.1, 148 USPQ 459 (1966)" This test includes evaluating any objective evidence of non-obviousness (i.e. so-called "secondary considerations"). In MPEP 1504.03, secondary considerations are exemplified by commercial success including evidence of extensive use by skilled artisans and unexpected results, both of which are applicable herein.

Double Patenting

Examiner Nashed asked that applicants amend claim 73 in the co-pending application or amend the claims objected to before the present application would be allowable.

This suggestion is improper in view of MPEP 1504.06 which states that

> If a provisional double patenting rejection (of any type) is the only rejection remaining in two conflicting applications, the examiner should withdraw that rejection in one of the applications (e.g. the application with the earlier filing date) and permit the application to issue as a patent.

The present case has an earlier filing date than the co-pending application.